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(71) Applicant: RUSH-PRESBYTERIAN-ST. LUKE'S MEDICAL CENTER [US/US]; 1653 West Congress Parkway, Chicago, IL 60612 (US).

(72) Inventor: COON, John, S. ; 324 South Humphrey Street, Oak Park, IL 60302 (US).

(74) Agents: JOHNSON, James, Dean et al.; Jones & Askew, 191 Peachtree Street, N.E., 37th Floor, Atlanta, GA 30303-1769 (US).

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(54) Title: METHODS AND COMPOSITIONS FOR REDUCING MULTI-DRUG RESISTANCE

(57) Abstract

The present invention comprises methods and compositions for reducing or eliminating multidrug resistance in cancers in humans or animals. According to the method and composition of the present invention, a non-ionic amphipathic ester of a fatty acid is administered to a patient in which a human or animal cancer exhibits multidrug resistance to the chemotherapeutic agent. The method and composition of the present invention may be employed with particular efficacy where multidrug resistance to any chemotherapeutic agent has been conferred upon a cancer.

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METHODS AND COMPOSITIONS FOR REDUCING MULTI-DRUG RESISTANCE

Technical Field

The present invention relates to the use of resistance modification agents *in vivo* to reverse multidrug resistance in human or animal tumor cells. More particularly, the present invention relates to the use of certain non-ionic surfactants comprising certain amphipathic esters of fatty acids as resistance modification agents.

Background of the Invention

One of the major problems of cancer chemotherapy is the existence of drug resistance in tumors resulting in reduced responsiveness to chemotherapy. Some human cancers, e.g. kidney and colon carcinoma, are drug resistant before treatment begins, while in others drug resistance develops over successive rounds of chemotherapy. One type of drug resistance, called multidrug resistance, is characterized by cross resistance to functionally and structurally unrelated drugs. Typical drugs that are effected by the multidrug resistance are doxorubicin, vincristine, vinblastine, colchicine and actinomycin D, and others. At least some multidrug resistance is a complex phenotype which has been linked to a high expression of a cell membrane drug efflux transporter called Mdr1 protein, also known as P-glycoprotein. This membrane "pump" has broad specificity and

acts to remove from the cell a wide variety of chemically unrelated toxins. (See Endicott, J.A., et al. "The Biochemistry of P-Glycoprotein-Mediated Multidrug Resistance", *Ann. Rev. Biochem.* Vol. 58, pgs. 127-71, 1989.)

Substances which reverse multidrug resistance are known as resistance modification agents (RMAs), and are of importance in potentiating the cytotoxicity of chemotherapeutic agents to which a human cancer has become resistant. Although many agents have been identified as RMAs *in vitro,* a large proportion have little or no therapeutic potential because of high toxicity *in vivo* at the doses required to reverse multidrug resistance. For example, metabolic poisons, such as azide, reverse multidrug resistance *in vitro* but have no usefulness *in vivo.* Most other highly effective RMAs, such as verapamil, appear to work as competitive antagonists of a drug binding site on the Mdr1 protein. Many of these agents also have toxicity which limits their usefulness *in vivo.* Consequently, there is a need to develop alternate pharmacological strategies for reversing multidrug resistance to provide RMAs with improved activity and lower overall toxicity.

Decreased intracellular drug accumulation through overexpression of the drug efflux Mdr1 protein is important to, but apparently not the only factor, in the multidrug resistance phenotype. Altered intracellular drug distribution and binding, among other possibilities, also seem to play a role. For example, the mechanism of reversing doxorubicin resistance using verapamil appears to be more related to altered intracellular distribution of doxorubicin than increased accumulation in the cell, as detailed in Schuurhuis, G.J., et al., "Quantitative determination of factors contributing to doxorubicin resistance in multidrug resistant cells," *J. Natl. Cancer Inst.*, 81:1887-1892, 1989. In that report, it is shown that doxorubicin is concentrated almost exclusively in the nucleus in drug sensitive cells, and mainly in the cytoplasm in drug resistant cells. With the addition of verapamil, doxorubicin is localized mainly in the nucleus in

drug resistant cells. Thus, high affinity binding of drugs to Mdr1 does not appear to be sufficient for optimal efflux, suggesting the existence of additional, rate limiting steps which may be susceptible to pharmacological intervention.

Certain non-ionic amphipathic surfactants, such as Tween 80 and Cremophor EL, have evidenced RMA activity. (See Riehm H., et al. "Potentiation of drug effect by Tween 80 in Chinese hamster cells resistant to actinomycin D and Danomycin" *Cancer Res.* Vol. 32, pgs. 1195-1200, 1972 and Woodcock, D. B., et al., "Reversal of the multidrug resistance phenotype with Cremophore EL, a common vehicle for water-insoluble vitamins and drugs" *Cancer Res.* Vol. 50, pgs. 4199-4203, 1990) However, Tween 80 potentiates drug toxicity in both parental and multidrug resistant cells, calling into question the specificity of the Tween 80 effect on multidrug resistance. An effect on drug efflux has not been demonstrated. Cremophor EL is a complicated mixture of polyoxyethylated esters of triglycerides of mainly ricinoleic acid (castor oil), the composition and active component of which have not been identified. Use of Cremophor EL *in vivo* is complicated by adverse histamine release in some patients.

Thus, what is needed is a clearly identified class of compositions that reverse multidrug resistance *in vivo*. The composition should have a low occurrence of adverse side-effects. The compositions should inhibit drug efflux by a mechanism different from antagonistic competition for a drug binding site on the Mdr1 protein, thereby broadening the pharmacological repertoire which may be employed to reverse multidrug resistance.

Summary of the Invention

The present invention comprises certain compositions that exhibit substantial RMA activity in cancers. One example of such a composition is a non-ionic amphipathic surfactant, known by the trade name SOLUTOL® HS 15 (BASF Corporation,

Parsippany, New Jersey). This composition increases the cytotoxicity of chemotherapeutic drugs in multidrug resistant cell lines, but not in drug sensitive cell lines, indicating that the potentiating effect is not due to the additive toxicity of the agent itself. The agent also promotes chemotherapeutic agent accumulation in multidrug resistant cells thereby potentiating the effect of the chemotherapeutic agent.

The present invention also comprises a method for reversing multidrug resistance in human or animal cancer cells and a composition for eliminating multidrug resistant human or animal cancer cells. One composition that is an aspect of the present invention is a particular fraction of SOLUTOL® HS 15 collected by reverse phase liquid chromatography. It has been found that the RMA activity in the SOLUTOL® HS 15 resides in a narrow fraction from the reverse phase liquid chromatography. It has been further determined that the toxicity to cells which is inherent in SOLUTOL resides in a fraction different from the fraction containing the RMA activity.

The present invention also includes a class of compounds which are ethoxylated fatty acids which exhibit strong RMA activity. These compounds have been found to be a fatty acid with between approximately 8 and 60 carbon atoms and between approximately 4 to 100 ethoxy units. The fatty acid component of the present invention can be unsaturated and can have one or more hydroxyl group. In general, the fatty acids without the ethoxy units have little or no RMA activity.

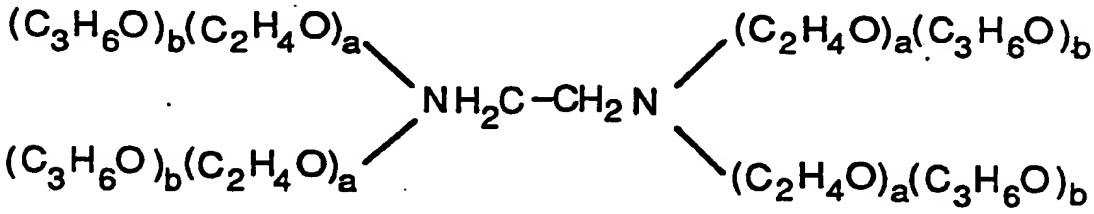
The present invention also includes compositions and methods for reducing the resistance of certain microorganisms to chemotherapeutic agents. It has been determined that certain microorganisms contain p-glycoprotein-like pumping mechanisms that are similar to those found in mammalian cells and it is believed that these mechanisms may be important in resistance to antmicrobial agents.

Another embodiment of the present invention are the polyoxyethylene/polyoxypropylene copolymers with the following general formula:

$$HO(C_2H_4O)_b(C_3H_6O)_a(C_2H_4O)_bH$$

wherein a is an integer such that the hydrophobe represented by (C_3H_6O) has a molecular weight of about 1200 to 9000, preferably 1750 to 4000, and b is an integer such that the hydrophile portion represented by (C_2H_4O) constitutes approximately 10% to 50% by weight of the compound.

Another embodiment of the present invention are the polyoxyethylene/polyoxypropylene copolymers with the following general formula:



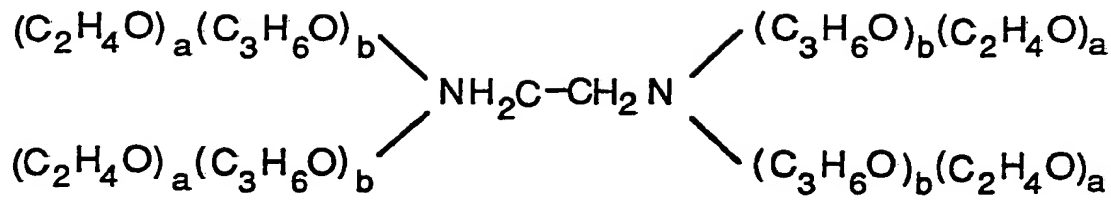
wherein:

the mean aggregate molecular weight of the portion of the octablock copolymer represented by the polyoxypropylene is between approximately 4500 and 7000 daltons;

a is a number such that the portion represented by polyoxyethylene constitutes between approximately 10% to 20% of the compound by weight, and;

b is a number such that the polyoxypropylene portion of the total molecular weight of the octablock copolymer constitutes between approximately 80% and 90% of the compound by weight.

Yet another embodiment of the present invention are the polyoxyethylene/polyoxypropylene copolymers with the following general formula:



wherein:

the mean aggregate molecular weight of the portion of the octablock copolymer represented by the polyoxypropylene is between approximately 4500 and 7000 daltons;

a is a number such that the portion represented by polyoxyethylene constitutes between approximately 10% to 40% of the compound by weight, and;

b is a number such that the polyoxypropylene portion of the total molecular weight of the octablock copolymer constitutes between approximately 60% and 90% of the compound by weight.

Accordingly, it is an object of the present invention to provide a composition and method for reducing or eliminating multidrug resistance in human or animal cancer cells.

It is further an object of the present invention to provide a composition and method for treating a human or animal with multidrug resistant cancer.

It is further an object of the present invention to provide a composition and method for reducing multidrug resistance which will not produce adverse side-effects.

It is further an object of the present invention to provide a composition and method that can be used to reduce the blood brain barrier thereby allowing certain therapeutic agents to cross the barrier from the blood into the brain.

It is yet another object of the present invention to provide a composition and method that can be used to reverse multidrug resistance to VP-16 and VM-26 in cancer cells.

It is yet another object of the present invention to provide a composition and method for reducing the resistance of microorganisms to certain drugs.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

Brief Description of the Figures

Figure 1 shows fractionation of SOLUTOL® HS 15 using reverse phase liquid chromatography.

Detailed Description

The present invention comprises methods and compositions for reducing or eliminating multidrug resistance in cancers in humans or animals. According to the method and composition of the present invention, a non-ionic amphipathic ester of a fatty acid is administered to a patient in which a human or animal cancer exhibits multidrug resistance to the chemotherapeutic agent. The method and composition of the present invention may be employed with particular efficacy where multidrug resistance to any chemotherapeutic agent has been conferred upon a cancer.

As used herein, the term multidrug resistance means resistance or acquired or natural resistance of tumor or other cells to chemotherapeutic agents. The multidrug resistance can be mediated by P-glycoprotein or can be mediated by other mechanisms.

The present invention includes a method of treating a human or animal with a cancer that exhibits multidrug resistance to reduce or eliminate the multidrug resistance which includes administering to the human or animal an effective amount of a non-ionic amphipathic ester of a fatty acid. A preparation that exhibits the desired biologic activity is SOLUTOL® HS 15. This

preparation is a mixture of various compounds with surfactant activities.

By fractionating the SOLUTOL® HS 15 preparation using reverse phase liquid chromatography and then assaying the various fractions for RMA activity, it has been determined that the RMA activity resides in a small fraction which contains fatty acid esters containing ethoxide units. This fraction has a much higher specific activity than the unfractionated SOLUTOL® HS 15. By synthesizing several fatty acid esters with varying ethoxide units, it has been found that compounds which are ethoxylated fatty acids exhibit strong RMA activity. These compounds have been found to be fatty acids or polymers of fatty acids with between approximately 8 and 60 carbon atoms and between approximately 4 to 100 ethoxy units. The fatty acid component of the present invention can be unsaturated and can be hydroxylated and still exhibit activity. In addition, the fatty acid can be branched. The preferred fatty acids are straight chained. In general, the fatty acids without the ethoxy units have little or no RMA activity.

The preferred compounds are fatty acids which have ethoxy units esterified on the carboxy group. The fatty acids have between 8 and 60 carbon atoms and between approximately 4 to 100 ethoxy units. If the fatty acid is hydroxylated the ethoxy units may be esterified at the hydroxyl group. The ethoxy units can be attached to the carboxyl group and/or the hydroxyl group if a hydroxyl group is present. The more preferred compounds have a fatty acid with between 12 and 50 carbons with the most preferred compounds with between 15 and 25 carbon atoms and between approximately 15 and 60 ethoxy units with the most preferred compounds having between approximately 15 and 20 carbon atoms. The preferred compounds have between approximately 4 and 100 ethoxy units, with the more preferred compounds having between 15 and 60 ethoxy units and the most preferred compounds having between 25 and 50 ethoxy units. Preferred fatty acids are selected from the group consisting of

stearic acid, 12-hydroxystearic acid, oleic acid, palmitic acid, and ricinoleic acid. The preferred number of ethoxy units are between approximately 5 and 50 units.

While not wanting to be bound by the following theory, it is believed that cellular membrane transport proteins must form polymers, usually dimers or tetramers, to effectively carry out their transport functions. Thus it is likely that the Mdr1 protein can achieve its function of removing from the cell a wide variety of chemically unrelated toxins only after forming polymers in the membrane. Non-ionic amphipathic surfactants exhibit membrane surface activity and are characterized by having a hydrophilic head and hydrophobic tail. In particular, non-ionic amphipathic esters of fatty acids, inhibit the formation of such protein polymers, and thereby inhibit drug efflux.

The ester of the present invention has a hydrophilic head, which comprises polyethylene glycol, and a hydrophobic tail comprising a fatty acid. Such a molecule is amphipathic: The molecule is large enough that each end displays its own solubility behavior.

The fatty acid component of the ester of the composition of the present invention can be selected from a wide range of fatty acids. It may advantageously possess at least one hydroxyl group outside of the carboxyl group. Such fatty acids can easily be esterified with themselves, as is well known in the art, to produce polymers of the fatty acid. For purposes of the present invention, the RMA can be formed not just from esters of a fatty acid monomer with polyethylene glycol, but such polymers of hydroxylated fatty acids also can be esterified with polyethylene glycol to form the RMA.

In a preferred embodiment of the present invention, the non-ionic amphipathic ester comprises polyethylene glycol ester of 12-hydroxystearic acid. Such a formulation is a component of a commercially available preparation from BASF Corporation (Parsippany, New Jersey) under the trade name SOLUTOL® HS 15.

The ester may be administered to a patient either alone or in combination with a treatment program of at least one chemotherapeutic agent to which the human cancer is resistant. Such a chemotherapeutic agent typically includes, but is not limited to, doxorubicin, vincristine, vinblastine, Taxol, colchicine, VP-16 and actinomycin D. However, there are many other chemicals used in chemotherapy to which multidrug resistance may appear during treatment, and the present invention may be employed equally well in such cases. In addition, the present invention is useful for reducing resistance to platinum compounds by promoting accumulation of these compounds.

In general, at least one effective dose of the RMA of the present invention is administered for every dose of chemotherapeutic agent that is administered in treatment. Preferably, an effective dose of the RMA may be administered at least daily throughout the period between administration of successive doses of chemotherapeutic agent. The treatment period typically lasts about four weeks, depending upon the cancer being treated and the chemotherapeutic agents being used. Alternatively, the RMA may be continuously infused throughout said period. The administration of the RMA may also commence prior to a session of chemotherapy, and continue throughout and after the chemotherapy session. The amount of the RMA per dose will depend on which particular non-ionic amphipathic fatty acid ester is employed according to the present invention. However it is preferable that the maximum dosage that may be tolerated with negligible toxic symptoms *in vivo* be used. At least some non-ionic amphipathic esters of fatty acids, such as SOLUTOL® HS 15, are tolerated extremely well *in vivo*, and may be employed with no acute toxicity at dosages which achieve equivalent or superior reversal of multidrug resistance to common chemotherapeutic agents as compared to dosages of the prototypical RMA verapamil which produce marked toxicity.

The RMA of the present invention can be administered either intravenously or orally. It may be

administered separately from the chemotherapeutic agent, as may be dictated by the chemotherapy, in which case the amount of time between commencing administration of the RMA and administration of the chemotherapeutic agent should not be substantial, e.g. typically within 24 hours, or as the chemotherapy permits. An exemplary treatment regimen comprises oral or intravenous administration of the chemotherapeutic agent, followed by continuous administration of the RMA throughout the period until the next session of chemotherapy, either by continuous infusion or oral time release capsules. A typical dose for a human of the SOLUTOL® HS 15 is between approximately 1 mg/kg and 250 mg/kg. A more preferred dose of SOLUTOL® HS 15 is between approximately 5 mg/kg and 100 mg/kg. If a purified esterified fatty acid is used to treat a human with multidrug resistant cancer, the preferred dose is between approximately 1 mg/kg and 200 mg/kg with the more preferred dose between approximately 15 mg/kg and 60 mg/kg.

Alternatively, the RMA of the present invention may be administered in combination with the chemotherapeutic agent, comprising continuous infusion or daily oral consumption of time release capsules of the RMA commencing prior to the chemotherapy session, and continuing throughout and after the session, by way of example. The RMA may be infused together through the same needle with the chemotherapeutic agent, or combined in a single oral capsule, as the chemotherapeutic agent permits, in which cases the RMA of the present invention may be used as an emulsifier of the agent, since non-ionic amphipathic esters of fatty acids commonly possess emulsifying characteristics.

Preparation of an emulsion of the chemotherapeutic agent with the RMA will depend on the particular agents used. Typically, the RMA and the chemotherapeutic agent are combined and heated above room temperature to a range in which both the RMA and the chemotherapeutic agent are still stable, but in which the RMA becomes fluid, about 50° to 80° C. Sterile water is heated to the same temperature and then added with vigorous

agitation in a proper amount to achieve a viscosity appropriate for administration. Other components may be added to the emulsion as necessary to prepare it either for intravenous or oral administration, as is well known in the art.

According to another embodiment of the present invention, the RMA of the present invention can be administered together with other RMAs, such as verapamil. The RMA of the present invention and a second RMA can be infused separately or concurrently, or combined into one time release capsule for oral consumption, in effective doses typically administered in treatment using each RMA alone, as permitted by the toxicity of the second RMA.

The method and composition of the present invention provide an important new means of overcoming multidrug resistance in human cancers. The method and composition have an efficacy equal to or better then best resistance modification agents known to the inventor. Furthermore the agent used in the method and composition of the present invention has a lower toxicity than other RMAs and fewer side effects than other potential RMAs. Moreover, it is believed that the agent operates by a different mechanism on the complex phenotype of multidrug resistance, and thus can be combined with other RMAs to provide a more potent means of reversing multidrug resistance.

The structure of SOLUTOL® HS 15 is dissimilar to that of verapamil or other typical RMAs. The markedly greater potency of SOLUTOL® HS 15 than verapamil for reversing VP-16 or colchicine resistance relative to the ability of each to reverse vinblastine or doxorubicin resistance supports the hypothesis that SOLUTOL® HS 15 operates by a MDR-reversing mechanism different from competition for the drug-binding site on Mdr1 protein found in verapamil. Colchicine is known to interact weakly with the identified drug-binding site on the Mdr1 protein, since colchicine does not compete for vinblastine binding. The fact that MDR cells are nevertheless highly resistant to colchicine indicates that colchicine efflux is less dependent on interaction

with this drug-binding site than is vinblastine. Since SOLUTOL® HS 15 is a highly potent RMA for both colchicine and vinblastine, it may inhibit a second event necessary for efflux after drug binding, namely actual transport through the membrane. It is likely that SOLUTOL® HS 15, as a surfactant, inhibits formation of Mdr1 protein polymers which may be necessary to achieve drug efflux.

Another important advantage of the RMA of the present invention is the fact that the compounds which are contemplated as part of the present invention are highly effective against the multidrug resistance against the anticancer drug VP-16. The prior art RMAs, such as verapamil, are not effective against VP-16 multidrug resistance. (See Schested, M, et al. "Relationship of VP-16 to the Classical Multidrug Resistance Phenotype", *Cancer Research*, Vol. 52, pgs. 2874-2879, 1992.) The RMAs of the present invention have been found to be effective in reducing multidrug resistance against a broad spectrum of anticancer drugs.

It is well known that certain microorganisms contain membrane proteins which are similar in structure and function to the P-glycoprotein that is expressed by the MDR1 gene in mammals. It is contemplated as part of the present invention that the methods and compositions that make up the present invention can be used to make certain microorganisms more susceptible to therapeutic drugs. For example, it is likely that the present invention will reverse chloroquine resistance in malaria.

Another embodiment of the present invention relates to the blood brain barrier. It has been reported that the P-glycoprotein pump exists in brain capillary endothelium. (See Tasuta, T., et al., Functional Involvement of P-glycoprotein in Blood-Brain Barrier", *J. Biol. Chem.*, Vol. 267, pgs. 20383-20391, 1992.) The brain is a pharmacologic sanctuary in that many drugs administered systemically have limited access to the tissue parenchyma. In the brain, endothelial cells forming the capillary tube are joined by continuous tight junctions that

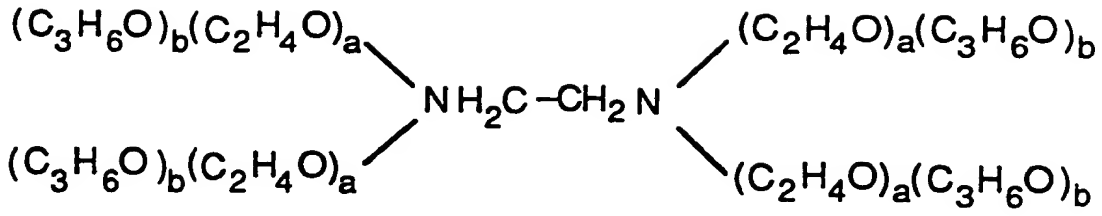
prevent many substances from entering the organ. Nutrients needed for brain cells are selectively transported from the blood through specific channels or transporters in the capillary endothelial cells. Thus, the brain is a rigorously isolated compartment that is protected by a blood-brain barrier. Hydrophobic antitumor agents, such as *Vinca* alkaloid and adriamycin (ADM), cannot enter the brain, although other hydrophobic molecules such as nicotine and ethanol readily pass through the blood-brain barrier. Therefore, some mechanisms of the barrier that selectively block the penetration of lipid-soluble antitumor agents into the brain could exist. The presence of P-glycoprotein in the capillary endothelium has been reported in both brain and testis but not in the other tissues. This suggests the functional involvement of P-glycoprotein in the blood-brain barrier. It is contemplated as part of the present invention that the methods and compounds described herein can be used to reduce the blood-brain barrier thereby allowing beneficial therapeutic agents to cross the barrier.

Another embodiment of the present invention are compounds that are effective in reducing multidrug resistance in cancer cells that are polyoxyethylene/polyoxypropylene copolymers with the following general formula:

$$HO(C_2H_4O)_b(C_3H_6O)_a(C_2H_4O)_bH$$

wherein a is an integer such that the hydrophobe represented by (C_3H_60) has a molecular weight of about 1200 to 9000, preferably 1750 to 4000, and b is an integer such that the hydrophile portion represented by (C_2H_40) constitutes approximately 10% to 50% by weight of the compound.

In another embodiment of the present invention, the block copolymer comprises a polymer of hydrophilic polyoxyethylene (POE) built on an ethylene diamine initiator. Polymers of hydrophobic polyoxypropylene (POP) are then built on the block of hydrophilic polyethylene (POE). This results in an octablock copolymer with the following general formula:



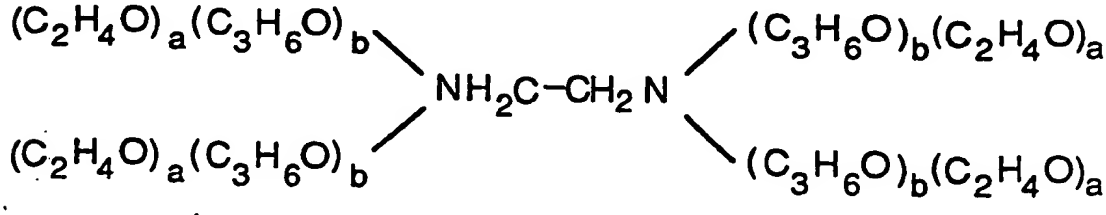
wherein:

the mean aggregate molecular weight of the portion of the octablock copolymer represented by the polyoxypropylene is between approximately 4500 and 7000 daltons;

a is a number such that the portion represented by polyoxyethylene constitutes between approximately 10% to 20% of the compound by weight, and;

b is a number such that the polyoxypropylene portion of the total molecular weight of the octablock copolymer constitutes between approximately 80% and 90% of the compound by weight.

In one embodiment of the present invention, the block copolymer comprises a polymer of hydrophobic polyoxypropylene (POP) built on an ethylenediamine initiator. Polymers of hydrophilic polyoxyethylene (POE) are then built on the block of hydrophobic polyoxypropylene (POP). This results in an octablock copolymer with the following general formula:



wherein:

the mean aggregate molecular weight of the portion of the octablock copolymer represented by the polyoxypropylene is between approximately 4500 and 7000 daltons;

a is a number such that the portion represented by polyoxyethylene constitutes between approximately 10% to 40% of the compound by weight, and;

b is a number such that the polyoxypropylene portion of the total molecular weight of the octablock copolymer constitutes between approximately 60% and 90% of the compound by weight.

The octablock copolymers comprising the biologically active copolymers of the present invention include, but are not limited to, the block copolymers Tetronic® and reverse Tetronic® manufactured by the BASF Corporation (BASF Corporation, Parsippany, NJ). The triblock copolymers are sold under the trademark PLURONIC® and are available from BASF Corporation.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

Example I

Human epidermoid carcinoma cell lines KB 8-5 and KB 8-5-11, which exhibit multidrug resistance, and their parental cell line KB 3-1, which is drug sensitive, were treated *in vitro* with SOLUTOL® HS 15 in combination with various chemotherapeutic agents, namely colchicine, vinblastine, and doxorubicin. The details of the treatment are described in Coon, J.S., et al, "SOLUTOL® HS 15, nontoxic polyoxyethylene esters of 12-hydroxystearic acid, reverses multidrug resistance", *Cancer Research*, 51, 897-902, 1991, which is incorporated by reference. Briefly, cells from the three lines were plated as is well known in the art in 96-well plates, with increasing concentrations of cytotoxic drug along one axis of the plate and increasing concentrations of the RMA along the other axis of the plate. After incubation for five days, the plates were washed and dyed according to methods known in the art, and a cell count was determined. The mean concentration of the cytotoxic drug that caused 50% inhibition of cell growth compared to controls (IC_{50}) was plotted at various concentrations of the RMA. Complete reversal of the MDR phenotype in KB 8-5 and KB 8-5-11 cells was achieved by SOLUTOL® HS 15, while the RMA did not potentiate drug toxicity in drug-sensitive KB 3-1 cells, indicating the potentiating effect was not due to any toxicity of SOLUTOL® HS 15 itself. At a concentration of 10% of its own IC_{50}, SOLUTOL® HS 15 produced a 35-, 28-, and 42-fold reduction in the resistance of KB 8-5-11 cells to colchicine, vinblastine, and doxorubicin, respectively.

Identical platings were also performed for the prototypical RMA verapamil. Unexpectedly, the relation between the effects that SOLUTOL® HS 15 had on the three cytotoxins was different from the relation between the effects that verapamil had on the three cytotoxins, indicating SOLUTOL® HS 15 and verapamil affect multidrug resistance by different mechanisms. SOLUTOL HS 15 was relatively much more potent than verapamil

for reversing colchicine resistance, as compared to the ability of each RMA to reverse vinblastine resistance.

Example II

Efflux of rhodamine 123 from MDR cells was also examined to provide direct information about the action of the transport protein Mdr 1. Briefly, prepared cells from the KB 8-5-11 line were washed and incubated in 0.5 µg/ml rhodamine 123 and 24 µM verapamil for 3 hours at 37° C. The cells were washed in ice cold DMEM, split into 3 aliquots, and incubated in either complete medium alone or complete medium with 24 µM verapamil or 70 µM SOLUTOL® HS 15 at 37°C. The rhodamine 123 fluorescence of the cells was measured periodically by flow cytometric analysis as described in Coon et al. The rhodamine 123 studies showed that SOLUTOL® HS 15 promotes drug accumulation in MDR cells, and furthermore that such accumulation is at least partly due to a pronounced decrease in the rate of drug efflux.

Example III

SOLUTOL® HS 15 was fractionated using reverse phase liquid chromatography to determine where the activity resides in the preparation. An approximately 50% solution of SOLUTOL® HS 15 was prepared in 100% acetonitrile (ACN) and water. One ml of the SOLUTOL® HS 15 solution was injected onto a Phenomenex IB-Sil reversed phase column. The column has 5 µm particles, and is 4.6 mm internal diameter by 150 mm. The flow rate was 2.0 ml/min. The mobile phase was as follows: A=5-% ACN and B=100% ACN. The gradient was linear with 100% A to 100% B in 15 minutes, then was maintained at 100% B. Fractions were collected at 30 second intervals. The various fractions were assayed for RMA activity as described in Example II. The results of the fractionation are shown in Figure 1. In addition the same fractions were assayed for toxicity by measuring 50% inhibitory concentrations (IC_{50}) as described in

Kessel D., "Exploring Multidrug Resistance using Rhodamine 123, *Cancer Communications* Vol 1, pgs. 145-149, 1989.

As can be seen in Figure 1, the RMA activity is confined in a single peak which elutes at approximately 20 minutes into the chromatographic run. The toxicity is confined to another peak that elutes before the activity peak and slightly overlaps the RMA peak. However, it is clear that most of the material that is responsible for the RMA activity is non-toxic.

Example IV

Using nuclear magnetic resonance spectroscopy and mass spectroscopy, the material that eluted under the activity peak in Figure 1 was analyzed, it is found that several species molecules are present. The molecules found under the activity peak in Figure 1 appear to be esterified fatty acids. To identify the chemical compounds having RMA activity, several fatty acid esters were synthetically prepared and tested for RMA activity according to Example II. Preparation of the ethoxified fatty acids is well known to those of ordinary skill in the art. Synthesis of fatty acids and their derivatives with ethylene oxide are described in Bares, et al. *Tenside Detergents*, Vol. 12, p. 155 1975 and Wrigley, A.N. *J. Amer. Oil Chemists's Soc.* Vol. 34, p. 39, 1957. All compounds were administered to KB8-5-11 (multidrug resistant) cells *in vitro* at 100 μg/ml in tissue culture medium at 37° C. Rhodamine 123 at 0.5 μg/ml was also present. The results of these measurements are shown in Table I.

Table I
Rhodamine 123 accumulation in KB8-5-11 (MDR) cells treated with ethoxylated fatty acids

Fatty Acid	Number of EO units	% Accumulation[a]
None[b]	-	3.2
SOLUTOL®	Mixture	99.6
Stearic acid	0	3.3
12-hydroxy stearic acid	0	3.1
Oleic acid	0	8.4
Ricinoleic acid	0	6.5
Stearic acid	5	7.2
Stearic acid	15	72.1
Stearic acid	45	99.8
12-hydroxy stearic acid	5	33.1
12-hydroxy stearic acid	15	35.3
12-hydroxy stearic acid	45	90.0
Oleic acid	5	3.6
Oleic acid	15	55
Oleic acid	45	99.9
Ricinoleic acid	5	25.9
Ricinoleic acid	15	nd
Ricinoleic acid	45	92.0

a. Per cent cells showing rhodamine 123 fluorescence in the range of sensitive cell (KB3-1) in the same experiment
b. For KB3-1 (sensitive) cells, 100.0% accumulated rhodamine
c. not done

Example V

Toxicity studies indicate SOLUTOL® HS 15 is extremely well tolerated *in vivo*. Pure-bred beagle dogs received intravenous doses of 5, 25, 50 or 100 milligrams of SOLUTOL® HS 15 per kilogram body weight, daily over a period of 4 weeks. No signs of toxicity were found in doses up to 25 mg/kg. At 50 mg/kg, sporadic and transient pruritus, erythema, and/or urticaria were observed. After doses of 100 mg/kg, the dogs showed different degrees of pruritus, erythema, or urticaria, most pronounced 5 to 10 minutes after injection, and no longer detectable after 60 minutes. These studies indicate SOLUTOL® HS 15 is better tolerated *in vivo* than Cremophor EL.

Example VI

The PLURONIC® and TECTRONIC'® copolymers were tested for RMA activity in a manner similar to that shown in Example IV. The results of these measurements are shown in Table II.

Table II
Rhodamine 123 accumulation in KB8-5-11 (MDR) Cells Treated with Polyoxyethylene/polyoxypropylene Block Copolymer

Compound	µg/ml (70µM)	% Accumulation[a]
None[b]	-	1.2
SOLUTOL®	100	72.3
Verapamil[c]	24µM	98.2
Benzyl alcohol[d]	5405 (50mM)	4.6
PLURONIC® COPOLYMERS		
F-38	329	0.1
F-77	462	1.1
L-81	192	97.4
L-101	270	21.0
F-108	1022	0.3
L-121	308	64.2
F-127	880	5.7
L-141	336	78.2
L-190.5	607	100.0
TETRONIC COPOLYMERS		
T1301	476	99.7
T1302	539	89.7
T1501	553	42.8
REVERSE TETRONIC COPOLYMERS		
T110R-1	360	100.0
T130R-1	476	97.3
T130R-2	542	50.2
T150R-1	50	87.1

a. Per cent cells showing rhodamine 123 fluorescence in the range of sensitive cell (KB3-1) in the same experiment
b. For KB3-1 (sensitive) cells, 99.3% accumulated rhodamine
c. Verapamil is a reversing agent
d. Non-specific membrane fluidizer

As can be seen in Table II, several of the polyoxyethylene/polyoxyethylene block copolymers are effective

in reducing multidrug resistance in cancer cells exhibiting the activity.

It should be understood, of course, that the foregoing relates only to a preferred embodiment of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

What Is Claimed Is:

1. A method of reversing multidrug resistance in a cancer in a human or animal with the cancer comprising the steps of administering to the human or animal an effective amount of a resistance modification agent comprising a non-ionic amphipathic ester of a fatty acid.

2. The method of Claim 1, wherein the ester comprises a hydrophilic head selected from the group consisting of polyethylene glycol and saccharides.

3. The method of Claim 2, wherein the polyethylene glycol has between approximately 4 and 100 ethylene oxide units.

4. The method of Claim 3, wherein the polyethylene glycol has between approximately 15 and 60 ethylene oxide units.

5. The method of Claim 4, wherein the polyethylene glycol has between approximately 25 and 50 ethylene oxide units.

6. The method of Claim 1, wherein the fatty acid is selected from the group consisting of saturated fatty acids, unsaturated fatty acids, hydroxylated fatty acids and hydroxylated unsaturated fatty acids.

7. The method of Claim 6, wherein the fatty acid is selected from the group consisting of stearic acid, 12-hydroxystearic acid, oleic acid, palmitic acid, and ricinoleic acid.

8. The method of Claim 6, wherein the fatty acid has between approximately 8 and 60 carbon atoms.

9. The method of Claim 8, wherein the fatty acid has between approximately 12 and 50 carbon atoms.

10. The method of Claim 9, wherein the fatty acid has between approximately 15 and 25 carbon atoms.

11. A method for potentiating the cytotoxicity of a chemotherapeutic agent in a human or animal with a cancer exhibiting multidrug resistance comprising the step of administering to the human or animal an effective amount of a resistance modification agent comprising a non-ionic amphipathic ester of a fatty acid in combination with a chemotherapeutic agent.

12. The method of Claim 11, wherein the ester comprises a hydrophilic head selected from the group consisting of polyethylene glycol and saccharides.

13. The method of Claim 12, wherein the polyethylene glycol has between approximately 4 and 100 ethylene oxide units.

14. The method of Claim 13, wherein the polyethylene glycol has between approximately 15 and 60 ethylene oxide units.

15. The method of Claim 14, wherein the polyethylene glycol has between approximately 25 and 50 ethylene oxide units.

16. The method of Claim 11, wherein the fatty acid is selected from the group consisting of saturated fatty acids, unsaturated fatty acids, hydroxylated fatty acids and hydroxylated unsaturated fatty acids.

17. The method of Claim 16, wherein the fatty acid is selected from the group consisting of stearic acid, 12-hydroxystearic acid, oleic acid, palmitic acid, and ricinoleic acid.

18. The method of Claim 17, wherein the fatty acid has between approximately 8 and 60 carbon atoms.

19. The method of Claim 18, wherein the fatty acid has between approximately 12 and 50 carbon atoms.

20. The method of Claim 19, wherein the fatty acid has between approximately 15 and 25 carbon atoms.

21. A composition for treating multidrug resistant human cancer cells in a human or animal comprising at least one non-ionic amphipathic ester of a fatty acid and at least one chemotherapeutic agent.

22. The composition of Claim 21, wherein the ester comprises a hydrophilic head selected from the group consisting of polyethylene glycol and saccharides.

23. The composition of Claim 22, wherein the polyethylene glycol has between approximately 4 and 100 ethylene oxide units.

24. The composition of Claim 23, wherein the polyethylene glycol has between approximately 15 and 60 ethylene oxide units.

25. The composition of Claim 24, wherein the polyethylene glycol has between approximately 25 and 50 ethylene oxide units.

26. The composition of Claim 21, wherein the fatty acid is selected from the group consisting of saturated fatty acids, unsaturated fatty acids, hydroxylated fatty acids and hydroxylated unsaturated fatty acids.

27. The composition of Claim 26, wherein the fatty acid is selected from the group consisting of stearic acid, 12-hydroxystearic acid, oleic acid, palmitic acid, and ricinoleic acid.

28. The composition of Claim 27, wherein the fatty acid has between approximately 8 and 60 carbon atoms.

29. The composition of Claim 28, wherein the fatty acid has between approximately 12 and 50 carbon atoms.

30. The composition of Claim 29, wherein the fatty acid has between approximately 15 and 25 carbon atoms.

31. The composition according to Claim 21 wherein the chemotherapeutic agent is selected from the group consisting of doxorubicin, daunomycin, vincristine, vinblastine, taxol, colchicine, VP-16, camptotechin and actinomycin.

32. A method of reversing multidrug resistance in a cancer in a human or animal with the cancer comprising the steps of administering to the human or animal an effective amount of a resistance modification agent comprising a polyoxyethylene/polyoxypropylene copolymer with the following general formula:

$$HO(C_2H_4O)_b(C_3H_6O)_a(C_2H_4O)_bH$$

wherein a is an integer such that the hydrophobe represented by (C_3H_60) has a molecular weight of about 1750 to 9000, and b is an integer such that the hydrophile portion represented by (C_2H_40) constitutes approximately 10% to 50% by weight of the compound.

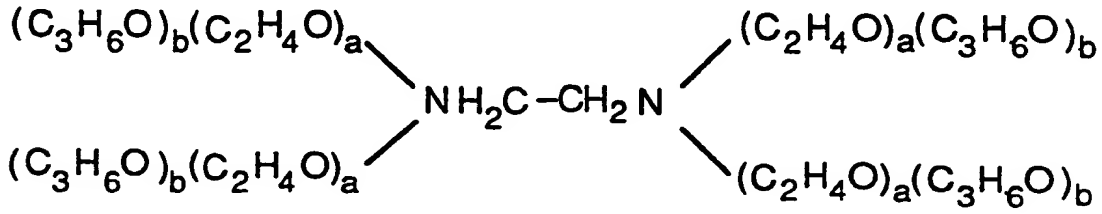
33. A composition for treating multidrug resistant human cancer cells in a human or animal comprising at least one chemotherapeutic agent and at least one polyoxyethylene/polyoxypropylene copolymer with the following general formula:

$$HO(C_2H_4O)_b(C_3H_6O)_a(C_2H_4O)_bH$$

wherein a is an integer such that the hydrophobe represented by (C_3H_60) has a molecular weight of about 1750 to 9000, and b is an integer such that the hydrophile portion represented by (C_2H_40) constitutes approximately 10% to 50% by weight of the compound.

34. The composition according to Claim 33, wherein the chemotherapeutic agent is selected from the group consisting of doxorubicin, daunomycin, vincristine, vinblastine, taxol, colchicine, VP-16, camptotechin and actinomycin.

35. A method of reversing multidrug resistance in a cancer in a human or animal with the cancer comprising the steps of administering to the human or animal an effective amount of a resistance modification agent comprising a polyoxyethylene/polyoxypropylene copolymer with the following general formula:



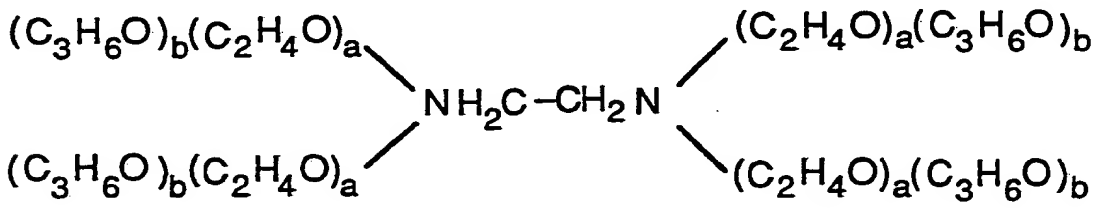
wherein:

the mean aggregate molecular weight of the portion of the octablock copolymer represented by the polyoxypropylene is between approximately 4500 and 7000 daltons;

a is a number such that the portion represented by polyoxyethylene constitutes between approximately 10% to 20% of the compound by weight, and;

b is a number such that the polyoxypropylene portion of the total molecular weight of the octablock copolymer constitutes between approximately 80% and 90% of the compound by weight.

36. A composition for treating multidrug resistant human cancer cells in a human or animal comprising at least one chemotherapeutic agent and at least one polyoxyethylene/polyoxypropylene copolymer with the following general formula:



wherein:

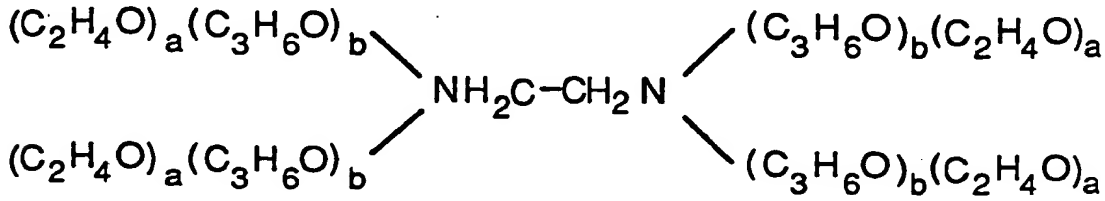
the mean aggregate molecular weight of the portion of the octablock copolymer represented by the polyoxypropylene is between approximately 4500 and 7000 daltons;

a is a number such that the portion represented by polyoxyethylene constitutes between approximately 10% to 20% of the compound by weight, and;

b is a number such that the polyoxypropylene portion of the total molecular weight of the octablock copolymer constitutes between approximately 80% and 90% of the compound by weight.

37. The composition according to Claim 36, wherein the chemotherapeutic agent is selected from the group consisting of doxorubicin, daunomycin, vincristine, vinblastine, taxol, colchicine, VP-16, camptotechin and actinomycin.

38. A method of reversing multidrug resistance in a cancer in a human or animal with the cancer comprising the steps of administering to the human or animal an effective amount of a resistance modification agent comprising a polyoxyethylene/polyoxypropylene copolymer with the following general formula:



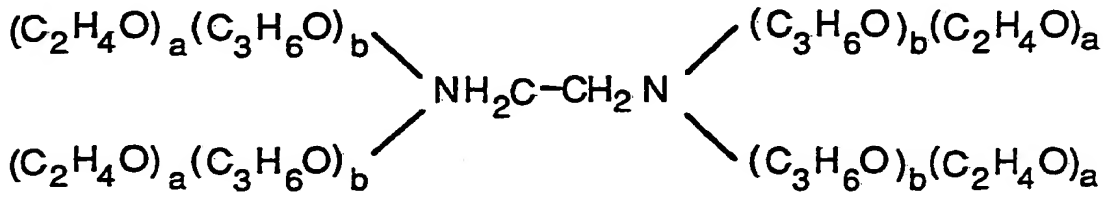
wherein:

the mean aggregate molecular weight of the portion of the octablock copolymer represented by the polyoxypropylene is between approximately 4500 and 7000 daltons;

a is a number such that the portion represented by polyoxyethylene constitutes between approximately 10% to 40% of the compound by weight, and;

b is a number such that the polyoxypropylene portion of the total molecular weight of the octablock copolymer constitutes between approximately 60% and 90% of the compound by weight.

39. A composition for treating multidrug resistant human cancer cells in a human or animal comprising at least one chemotherapeutic agent and at least one polyoxyethylene/polyoxypropylene copolymer with the following general formula:



wherein:

the mean aggregate molecular weight of the portion of the octablock copolymer represented by the polyoxypropylene is between approximately 4500 and 7000 daltons;

a is a number such that the portion represented by polyoxyethylene constitutes between approximately 10% to 40% of the compound by weight, and;

b is a number such that the polyoxypropylene portion of the total molecular weight of the octablock copolymer constitutes between approximately 60% and 90% of the compound by weight.

40. The composition according to Claim 39, wherein the chemotherapeutic agent is selected from the group consisting of doxorubicin, daunomycin, vincristine, vinblastine, taxol, colchicine, VP-16, camptotechin and actinomycin.

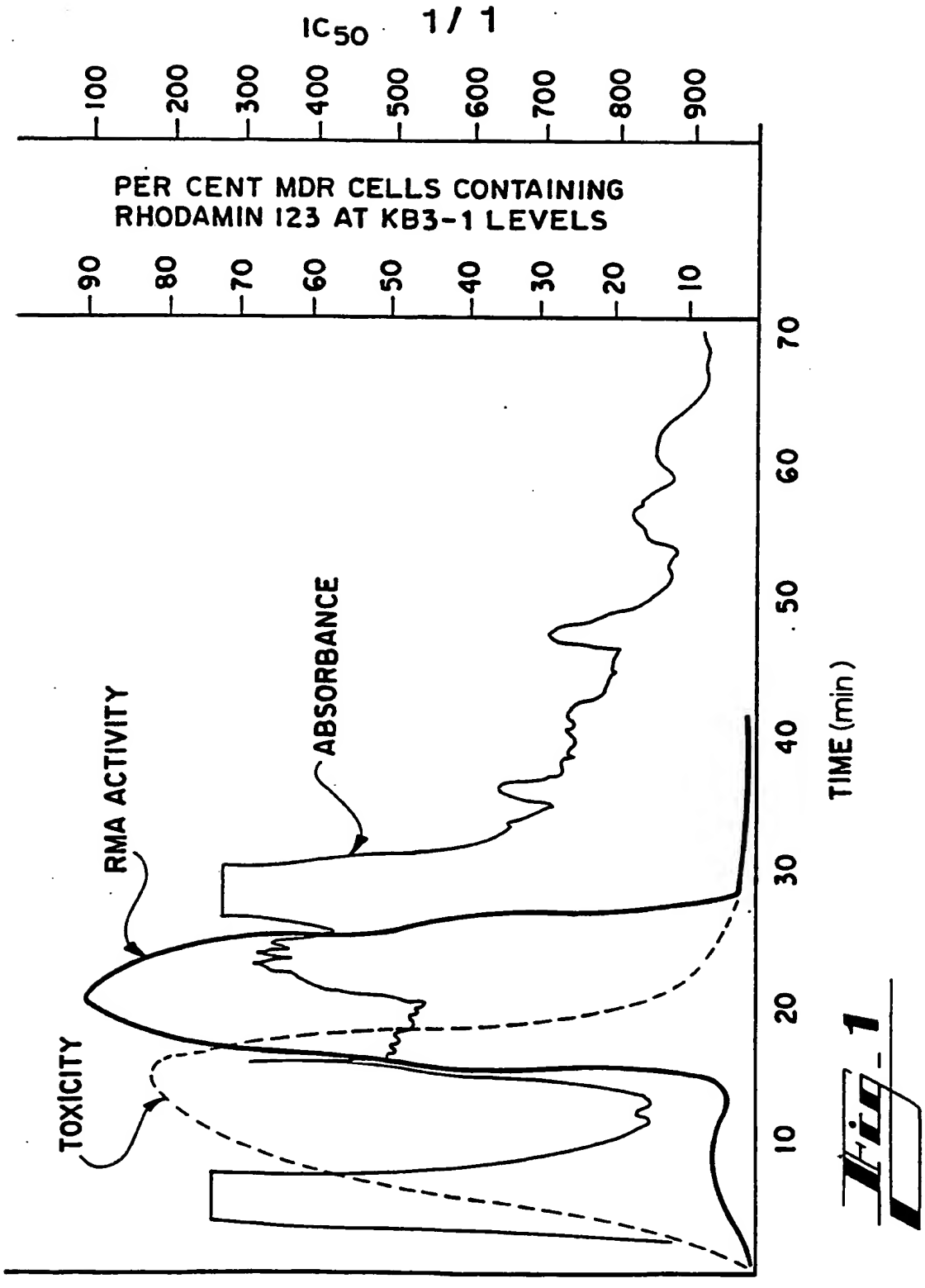


Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10563

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A01N 43/04, 43/38, 43/42; A61K 31/33, 31/40, 31/42, 31/70
US CL :514/10, 34, 35, 183, 283, 411, 506, 515, 765, 950
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/10, 34, 35, 183, 283, 411, 506, 515, 765, 950

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US,A, 3,919,411 (GLASS) 11 November 1975 See the abstract.	21-31 33,34,36,37,39,40
X Y	US,4,557,934 (COOPER) 10 December 1985 See the abstract.	21-31 33,34,36,37,39,40
X Y	US,A, 4,563,351 (CASLAVSKY) 07 January 1986 See the entire document.	21-31 33,34,36,37,39,40
X Y	US,A, 4,753,965 (STEMERICK) 28 June 1988 See the entire document.	21-31 33,34,36,37,39,40

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search
05 FEBRUARY 1993

Date of mailing of the international search report
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Authorized officer
NATHAN M. NUTTER for
Telephone No. (703) 308-2351

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10563

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US,A, 4,803,081 (FALK) 07 February 1989 See the entire document.	21-31 33,34,36,37,39,40
X Y	US,A, 4,863,968 (EDWARDS) 05 September 1989 See the entire document.	21-31 33,34,36,37,39,40
X Y	US,A, 4,889,525 (YUHAS) 26 December 1989 See the entire document.	21-31 33,34,36,37,39,40
X Y	US,A, 4,904,697 (SUNKARA) 27 February 1990 See the entire document.	21-31 33,34,36,37,39,40
X Y	US,A, 4,923,862 (HIROTA) 08 May 1990 See the entire document.	21-31 33,34,36,37,39,40
X Y	US,A, 4,978,332 (LUCK) 18 December 1990 See the entire document.	21-31 33,34,36,37,39,40
X Y	US,A, 4,978,622 (MISHELL) 18 December 1990 See the entire document.	11-31 33,34,36,37,39,40
X,P Y	US,A, 5,108,989 (AMENTO) 28 April 1992 See the entire document.	11-31 33,34,36,37,39,40
Y	COON "Solutol HS 15, Nontoxic Polyoxyethylene Esters of 12-Hydroxystearic Acid, Reverses Multidrug Resistance" Cancer Research, Vol. 51, pages 897-902, published 01 February 1991. See the Abstract and Results sections.	1-10,21-40
Y	LELONG "Fluorescent Verapamil Derivative for Monitoring Activity of the Multidrug Transporter" Molecular Pharmacology, Vol. 40, pages 490-494. Published 28 June 1991. See the Summary.	1-10,21-40
Y	RIEHM "Potentiation of Drug Effect by Tween 80 in Chinese Hamster Cells Resistant to Actinomycin D and Daunomycin" CANCER RESEARCH, Vol. 32, pages 1195-1200. Published June 1972. See the entire document.	11-31,33,34,36,37,39,40

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10563

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WOODCOCK "Reversal of the Multidrug Resistance Phenotype with Cremophor EL, a Common Vehicle for Water-insoluble Vitamins and Drugs" Cancer Research, Vol. 50, pages 4199-4203. Published 15 July 1990. See the entire document.	1-10,21-40

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10563

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

CAS ONLINE, BIOSIS, USPTO APS
(amphiphathic or amphi(w)pathic or tween or amphiphile or amphilic or cremophor or solutol) and (chemotherap? or doxorubicin or ?daunomycin or adriamycin or adriablastin? or vineristine or leukocristin? or vinblastin? or leukoblastin?)